("high" tau=28.43 ± 3.3; "low" tau=22.2 ± 1.08). Once we had the Tcrit (20.55 ms) we obtained the rate constants for intraburst activity with a C1-O-C2 model: k 1-2 = 182.44 ± 24.98; k 2-1 =4.57 ± 2.4; k 2-3 = 196.33 ± 32.85; k 3-2 = 4418.64 ± 299.37 (n = 8). Finally we studied the effects of chlorpromazine, a known modulator of mechano-activated channels, on these rate constants and found a decrease in the k 3-2/k 2-3 relationship from 22.97 ± 3.39 to 10.64 ± 1.47 ($p<0.05$; n=6). This model allows for formal kinetic studies of this novel channel.

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Mechanisms Underlying the Loss-Of-Functional Kir6.1 KATP Channel Mutations in Sudden Infant Death Syndrome

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INTRODUCTION: Hypoxia-induced apoptosis and arrhythmia are the important cause of sudden infant death syndrome (SIDS). ATP-sensitive K^+ (KATP) channels are known to provide a functional linkage between the electrical activity of cell membrane and metabolism. KCNJ8-encoded Kir6.1 KATP channel critically regulates vascular tone and cardiac adaptive response to systemic metabolic stressors, including sepsis. Previously, we identified two KCNJ8 mutations (E332del and V346I) in a large SIDS cohort that exhibited a marked loss-of-function phenotype and reduction of cell surface expression. Here we further investigate the mechanisms underlying the loss-of-functional Kir6.1 KATP channel mutations in SIDS.

Methods and Results: A hemagglutinin (HA) epitope was inserted in an extracellular loop of Kir6.1 wild type (WT), Kir6.1-E332del and Kir6.1-V346I channels. HEK293 cells were co-transfected with cDNA encoding HA-tagged Kir6.1-WT, HA-tagged Kir6.1-E332del or -V346I and SUR2A in a ratio of 1:1:2. Cell surface expression was assessed by Flow-cytometry with FITC-conjugated anti-HA antibody. Apoptosis assays were performed on HEK293 cells transfected with IRES-GFP constructs containing Kir6.1-WT or mutant (E332del or V346I) with SUR2A. After staining with PE-Annexin V and 7-AAD, the apoptotic cells were measured by Flow-cytometry within gated GFP (+) cells. Caspase-3/7 activity was measured with Apo-ONE Homogenous caspase 3/7 assay kit. The cell-counting studies showed that the cell surface expression of Kir6.1-WT was suppressed 40% to 70% when co-expressed with Kir6.1-E332del or Kir6.1-V346I. The apoptosis assay data indicated that the apoptotic ratio was increased significantly for E332del (38.9%) compared to WT (4.02%) and for V346I (11.2%) compared to WT (3.04%). The Caspase-3/7 activity was also increased 2.1 fold for E332del and 1.6 fold for V346I over WT.

Conclusions: The loss-of-functional Kir6.1 KATP channel mutations found in SIDS display a dominant-negative effect on Kir6.1-WT channels and induce apoptosis in heterologous expression system.

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Studying Clustering of KcsA Channels using Single-Channel Voltage-Clamp Fluorescence Imaging

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Protein oligomerization lies at the core of numerous biological processes, from cellular signal transduction to muscle contraction and cellular metabolism. It also notably drives the function of ion channels, as several subunits are often required to stabilize and gate specific ions through the conducting pore. In addition to these protein-protein interactions within ion channels, oligomerization or "clustering" of several ion channels has been observed. Although ion channel clustering is not generally thought to be a prerequisite for their physiological function, it has been suggested to modify the function of several ion channels. In this study, we optically observed clustering of single KcsA (E71A mutant) channels in planar lipid bilayer using single molecule fluorescence, while simultaneously measuring single channel currents. We found that clustering was not caused by direct protein-protein interactions but was mediated via microdomains induced in the lipid matrix. Interestingly, while KcsA clusters remained in the lipid bilayer, cooperative gating events with conductance levels multiple to the "normal" single channel events were often recorded. These coupled events were also observed in absence of a negatively charged phospholipid, which is believed to be required for KcsA activity. To understand the role of microdomains in coupled activity, we explored the physical properties of the lipid which could promote channel opening. Our findings show that lipids able to produce negative curvature in the lamellar liquid crystal phase ($L\alpha$) can induce channel activity, even without any negative charged lipid. We propose that the lateral pressure distribution of such lipid on the channel supports channel opening. This idea is in line with the cooperative gating of KcsA in the presence of clusters. The assembly of oligomers helps to overcome the energy barrier for opening by distributing the lateral pressure more favorably.

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Crystal Structure of Fluc, a Microbial Fluoride Channel

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Widespread bacteria, archaea, unicellular eukaryotes, and plants possess fluoride channels, called Flucs, to export toxic environmental fluoride anion from the cytoplasm. These proteins conduct F- ion at ~10 pS, are >10,000-fold selective for fluoride over chloride, and are constructed as extremely unusual antiparallel dimers. Here, we present the x-ray crystal structure of a Fluc channel from Bordetella pertussis, in complex with a pore-blocking FN3 domain "monobody." The structure reveals a closed conformation of the protein with the predicted antiparallel architecture and an occluded hourglass-shaped pore at the subunit interface.

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Two-Sided Simultaneous Block of a F- Channel (FLUC)

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The recently discovered family of F- channels, the Flucs, are highly selective for F- and function to rescue microorganisms from F- toxicity in acidic environments. Structurally, Fluc is a four pass transmembrane protein that assembles as a dual-topology homodimer. This architecture, where two Fluc subunits orient antiparallel with respect to each other, is unique among ion channels and requires a two-fold symmetry axis parallel to the membrane plane. The symmetry axis further suggests that Fluc may present identical interfaces on both sides of a membrane.

In fact, using fibronectin III domain "monobody" blockers specifically selected from a phage display library for nano-molar binding affinity, it was shown that a single Fluc channel is blocked on both sides of a planar lipid bilayer [Stockbridge et. al. Nature Comm., in press]. This leaves open the question of whether the sites of block on either side of the channel can be occupied simultaneously. We approach this question by experimentally testing a two site block model O <=> B1 <=> B2 by analysis of monobody dependent block times in single channel electrophysiology recordings. In the case of symmetric block, this scheme postulates that the dwell times of the B1 and B2 composite blocked state should exhibit monobody concentration dependence. In addition, information about cooperativity, whether negative or positive, between the two blocking sites can be quantitatively determined.

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Regulation of ClC-3 Cl^-/H^+ Transport and "Gating" Transients by Chloride Pathway Residues and External Protons

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We investigated ClC-3 ion transport properties by employing a "ClC-5/3" plasmid, consisting of the ClC-5 N-terminal (M1-A46) linked to the core ClC-3 protein. This ClC-3 protein is redirected to the plasma membrane of HEK cells. Functionally it exhibits rapidly activated, outwardly rectifying sustained ion currents (I_{SS}) representing coupled Cl^-/H^+ transport, and prominent "on/off" transient gating charge (Q) movements, as recently described for a ClC-3 mutant (Guzman et al, 2013, ACS Chem Neurosci). Interpreting Q to represent movements of unprotonated (ie, incompletely cycling) "Glu_{ext}"/E224, ClC-3 exhibits low transport efficiency compared to ClC-5 or ClC-4, which exhibit larger I_{SS} and much smaller Q. Replacement of external Cl^- with SCN^- increases ClC-3 currents by 3-4 fold, and reduces H^+ coupling by ~90%. Removal of a conserved tyrosine (Y630S, V) positioned at the intersection of the Cl^-/H^+ pathways (Accardi et al, 2006, JMB) greatly increases currents and decreases Q. For Y630S, H^+ coupling is reduced by ~50%; SCN substitution decreases rather than increases current, and eliminates H^+ transport. An M568A mutant moderately impacts transport, increasing currents and decreasing Q by <50%. Reduction of external pH (pH 6 or 5) weakly inhibits ClC-3 I_{SS}, but markedly reduces Q, and shifts the Q(V) relationship toward more positive voltages. These results are consistent with external protons inhibiting cycling by neutralization of Glu_{ext}. An endogenous Cl^- current activated at pH5 (I_{acid}) has slow activation kinetics and lacks gating transients, is